

“ARBUSCULAR MYCORRHIZAL FUNGAL ASSOCIATION WITH DIFFERENT CULTIVARS OF LINSEED IN DROUGHT PRONE AREA OF SATARA DISTRICT (M.S), INDIA.”

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ABSTRACT

The present study was conducted to deal with the diversity of Arbuscular Mycorrhizal Fungi in the rhizosphere soil of Linseed cultivars namely ALSI-2, PKV-NL-260 and JLS-73 from drought prone area of Satara district (M.S), India. Linseed (*Linum usitatissimum* L.) is one of the important rabbi oilseed crops of India belongs to Linaceae family and genus *Linum*. In India, *Linum* is grown primarily for Linseed oil which is high source of omega-3 fatty acid not only used for human consumption but also for commercial use. The results revealed variation in soil pH, Arbuscular Mycorrhizal Fungal Spore number, root colonization percentage (%) and Arbuscular Mycorrhizal fungal species in different sampling sites and varieties. Maximum numbers of AM Fungal spores were 105 per 50 gm soil and highest root colonization percentage 86.66 % was recovered from rhizospheric soil from variety PKV-NL-260 with soil pH 8. In general, five species of AM Fungi belonged to four genera viz, *Glomus*, *Acaulospora*, *Scutellospora* and *Gigaspora* were characterized, being those of the genus *Glomus* was most predominant in rhizosphere soil of Linseed in all the sites and varieties. In conclusion, the study provided useful information to understand the AMF species in Linseed soil and revealed that soil pH, drought condition (high temperature) and geographical distribution were triggered AMF diversity and spore count in the rhizosphere of *Linum*.

Keywords : AM Fungi, Rhizospheric soil, Varieties, Root colonization, Spore density.

Introduction

Linseed (*Linum usitatissimum* L.) is one of the important rabbi oilseed crops of India belongs to Linaceae family and genus *Linum*. In India, *Linum* is grown primarily for Linseed oil which is not only used for human consumption but also for commercial use. Linseed oil is high in omega-3 fatty acid, which lowers cholesterol, in the diet. Flax seed is also used as proteinaceous feed for livestock as it contains 3 per cent oil and 36 per cent protein.

Presently the area under Linseed in India is 4.6 lakh ha with a production of 1.73 lakh tonnes and the average yield is 416 kg per ha which is less than world's average yield (720 kg/ha). The area under Flax seed is increasing every year but seed production of this crop is very less. Most soil upon which crop is grown are generally poor in nutrients. Therefore, sound management of soil and other resources play key role in productivity. In recent years, more emphasis is being given by the Government of India to boost production of oil seed.

Arbuscular Mycorrhizal Fungi are a group of microbes which form symbiotic associations with a wide range of plant species that enhance plant nutrition and growth (Torres-Ariasa *et al.* 2017). AM Fungi are major component of rhizosphere microflora in natural ecosystems and play significant role in the re-establishment of nutrient cycling (Peterson *et al.* 1984). Arbuscular mycorrhizal (AM) fungi are known to be well distributed throughout both hemispheres. These fungi can be isolated from a wide variety of natural habitats and are particularly abundant in cultivated lands. AM (Arbuscular Mycorrhizal) fungi are common inhabitants of the roots of several plants and the role of mycorrhizal associations in the mobilization and uptake of phosphorus and productivity of many leguminous and other crops is well documented. AM fungi also improve the soil quality and increase in uptake of P, N, K, Zn, S, Fe, Mg, Ca and Mn (Sundar *et al.* 2010). Diversity and dynamics of AM fungi in different host plant species and soil types of particular agro-climatic zone is very important in order to evaluate the natural status of their fungi in that region. Survey of literature do not show any report on association of AM fungi with *L. usitatissimum* L. in this area.

Hence, the present study has been undertaken in an attempt to evaluate the quality and quantity of AM Fungi along a site grown *Linum usitatissimum* L. from Dahiwadi.

Materials and Methods

Study site

The site experiments were conducted at the end of growing season 2018-19 of crop. The study site lies in eastern part of Satara district. Dahiwadi, locate on the Satara-Pandharpur highway and 86 kms from Satara. Geographically it lies 17° 42' 14.4216'' N latitude and 74° 32' 41.5248'' E longitude. Average rainfall annually is around 2-3 cm. It is drought prone region. Majority of the agricultural systems depend on monsoon, except few regions are well facilitated with Bore water and tube well irrigation. As the region identified as drought prone, the major food crops of this region are Bajara, Sorghum, Wheat and few other vegetable crops.

Collection of soil samples

Soil and plant root systems were sampled at the end of the growing season 2018-19. The samples were taken after removing the top litter layer (5–10 cm) and digging out an appropriate amount of soil close to the roots of the host plant from a depth of 10–15 cm. The samples were collected in triplicate in polythene bags, from different sites. The soil samples were stored at 4°C in the laboratory to sustain the viability of Arbuscular Mycorrhizal spores and to lower the activity of the rhizosphere micro flora.

Soil particles attached to fine feeder roots were removed by generous shaking. AM spores were isolated by the wet-sieving and decanting method of Gerdemann and Nicholson (1963).

The number of spores per 50 gm soil was counted under Olympus Binocular research microscope on Whatman filter paper No.1. They were mounted on the slide in PVLG (Poly Vinyl alcohol Lacto Glycerol). All the slides were observed under Olympus Trinocular research microscope (Model no. CH-20iTR). Spores were photographed using Digital camera (Canon A 640). The species level identification of different native AM fungi was done following the keys provided by Schenck and Perez (1990) and Rodrigues and Muthukumar (2009). The soil pH of different soils was measured using digital pH meter. The root samples were gently washed under tap water, softened with 10 % potassium hydroxide (KOH), acidified with 1N hydrochloric acid (HCL) and stained with 0.05% trypan blue in lactoglycerol at 4°C following the method of Phillips and Hayman (1970). Quantification of root colonization of the Arbuscular Mycorrhizal Fungi was carried out using following formula proposed by Giovannetti and Mosse (1980).

$$\text{Root colonization percentage (\%)} = \frac{\text{No. of segment colonized with AMF}}{\text{No. of segment observed}} \times 100$$

Results and Discussion

Three varieties namely, ALSI-2, PKV-NL-260 and JLS-73 were assessed for AM fungi dynamics from *Linum usitatissimum* L. crops belonging to Linaceae family. The spore count of all the soil samples was done per 50 gm of rhizospheric soil. Table no. 1 reflects the data of AMF (Arbuscular mycorrhizal fungi) spore population, percentage of root colonization with its soil pH at rhizosphere of *Linum usitatissimum* L. in three different varieties. All the site soils investigated in this study were diverse in nature.

Soil pH :

Soil pH ranges from acidic soil pH 6.5 to alkaline soil pH 8. The data indicates the percentage of root colonization and number of AMF spore accumulated in rhizospheric soils from various sites ranging from 53.3 to 73.33% and 89 to 105 spores per 50 gm of soil respectively.

AMF Spore Density:

Due to ubiquitous nature of Arbuscular mycorrhizal fungi they occurred in almost all soil samples, but the quality and quantity varied. In the RS (rhizospheric soil) of Linseed collected from three different varieties, maximum number of AMF spores (105 spores per 50 g soil) were recorded in the RS of Linseed from site second, variety PKV-NL-260 followed by RS of Site three, variety JLS-73 (95 spores per 50 g soil) and minimum (89 spores per 50 g soil) spores were reported from the RS of site one, variety ALSI-2 (Table 1) with soil pH 8.5, 7.4 and 6.2 respectively. These data are consistent with that reported by other authors such as García-González et al. (2016) the physicochemical characteristics of soils can generate variations in the number of spores. In the present study, intence Arbuscular mycorrhizal colonization was restricted in the epidermal and cortical parenchymatous cells near the root bases.

AM fungal colonization and Percentage (%) of root colonization:

The results in percentage (%) of the root colonization and percentage (%) of root length colonization were analyzed from soybean five varieties collected from the different five study sites (Table 1). The highest % root colonization and spore density were recorded in PKV-NL-260 as well as the lowest

in the ALSI-2 variety. The highest root colonization was found in the PKV-NL- 260 variety while the lowest was in ALSI-2. The maximum % of root colonization recorded in variety PKV-NL-260 was 86.66 %, followed by 73.33 % from site three variety JLS-73 and the Lowest % of root colonization found in a variety of ALSI-2 was 53.33 %. Root colonization vesicular, arbuscular and hyphal were recorded in this season (Table 1).

Table 1. AMF (Arbuscular mycorrhizal Fungi) spore population, percentage of root colonization and soil pH at *Linum usitatissimum* L. rhizospheric soil of three different sites and varieties.

Sites	Varieties	Soil pH	Number of AMF Spores per 50 gm of Rhizosphere Soil	AM fungal colonization			Percentage of root colonization (%)
				Hyphae	Arbuscles	Vesicles	
1	ALSI-2	6.5	89	+	+	-	53.33
2	PKV-NL-260	8	105	+	+	-	86.66
3	JLS-73	7.2	95	+	-	+	73.33

Colonization was characterized by the presence of hyphae, arbuscules and vesicles. AM fungal colonization varied with their occurrence in three different sites. Likewise highest percentage of root colonization 86.66 % was reported from site second, followed by 73.33 % from site three and lowest percentage of root colonization 53.33 % was recorded from site one (Table 1). According to Majewska et al. (2018) and Mane, P. A. and Khilare C. J. (2014) both native and non-native plant species influence the physical and chemical properties of the soil and thus the colonization of AMF. Arbuscular mycorrhizal fungal spore count and root colonization percentage was found higher in soils with higher pH values. These findings were corroborated with the findings of Goransson et al., (2008) and Mane, P. A. and Khilare C. J. (2020)b that mycorrhizal spore count is reduced in acidic environment. There was a variation in the root colonization percentage (%) and number of AMF spores with soil pH in rhizospheric soils of plants from three different sites.

In the present piece of work, rhizosphere soil samples were screened for isolation of AM fungi (Gerdemann and Nicolson, 1963) from three localities of Linseed crops. In Site one, four AMF species assignable to three genera were isolated from rhizosphere. In Site second, three AM species belonging to three genera were reported in rhizosphere of Linseed. Also in site three, five AM species belonging to four genera were reported in rhizosphere. The plants were found to be infested with various genus and species of AM Fungi.

Table 2 Occurrence of different Arbuscular mycorrhizal fungi in the rhizosphere of *L. usitatissimum* L. from three different varieties

Sr. No.	AM Fungal species	Occurrence of AMF		
		ALSI-2	PKVNL-260	JLS-73
1	<i>Acaulospora tuberculata</i>	-	+	+
2	<i>Gigaspora decipiens</i>	-	+	-
3	<i>Glomus fasciculatum</i>	+	+	+
4	<i>G. mosseae</i>	+	+	+
5	<i>Scutellospora auriglobosa</i>	+	-	+

RS= Rhizosphere site, NRS= Non-rhizosphere site, + =Present, - = Absent

Five species from four genera were reported from Dahiwadi area Dist. Satara (M.S.). The genus *Glomus* was dominant with two species *Glomus fasciculatum* [*Rhizophagus fasciculatus*] (Thaxt.) Gerd & Trappe and *G. mosseae* [*Funneliformis mosseae*] (T. H. Nicolson & Gerd.) Gerd. & Trappe. Some other studies done by Mane, P. A. and Khilare C. J. (2020)a and Qin et al. (2015) also found the predominant abundance of genus *Glomus* in agricultural soils. One species each of genera *Acaulospora*, *Gigaspora* and

Scutellospora was reported which was least abundant; these are *Acaulospora tuberculata* Trappe, *Gigaspora decipiens* T. H. Nicolson & N. C. Schenck and *Scutellospora auriglobosa*, Walker C. and Hall. In present study it was observed the genus *Glomus* was species wise rich, Chaudhary and Singh (2015) have reported the genus *Glomus* occupying more than 50% of total AMF spore count and distributed in all types of agriculture soils of India.

It is apparent from the present investigation; in the rhizosphere of Linseed crop plant AM fungi such as *Acaulospora tuberculata*, *Glomus fasciculatum* and *Scutellospora auriglobosa* were dominant. *G. mosseae* was occurred as subdominant. However, *Gigaspora decipiens* was showed their normal appearance (Table 2).

Despite the fact that the Genus *Glomus* was the dominant among all the sites and the Arbuscular mycorrhizal fungi diversities in the rhizosphere soil of sampling sites significantly differed (Table 2). We also found that AM fungal diversity was lowest in the agriculture soils, as has been found in other studies of Muchane et al (2012). The crops from three different sites were observed to be diverse in percentage of root colonization and AMF spore number at all the three rhizospheres. The predominance of *Glomus* species under different soil types might be due to the fact that they are widely adaptable to the varied soil properties and survival in both acidic and alkaline soils (Pande and Tarafdar 2004).

There was variation in the diversity and distribution of Arbuscular Mycorrhizal Fungi from crops rhizosphere from different sites. The acidic to alkaline pH of black soil was due to soil contains high exchangeable mineral elements at surface soil and moisture content. Soil quality means the capacity of soil to interact with biotic and abiotic component for maintaining its physico-chemical nature, biological produce and painting the plant health.

Present study revealed that the different agro ecosystems, display different relationship with AM fungi and influence differentially their qualitative and quantitative status.

Conclusions

In this study, Arbuscular Mycorrhizal fungi dynamics was investigated along a field grown *Linum usitatissimum* L. from drought prone area of Satara districts (M.S) India. Three sites and varieties of soil samples were rich in AM fungal spores. A total of five species was identified from four genera, with majority of genus *Glomus* in all the sites. We concluded that, the soil pH and different cultivation practices significantly influenced the AMF spore count and diversity in rhizospheric soils. The data generated not only provides us useful information to understand the native Arbuscular mycorrhizal fungal species diversity in oil yielding crop *Linum usitatissimum* L. rhizosphere but also revealed soil chemical properties and agricultural practices triggered abundance and root colonization percentage (%) of Arbuscular Mycorrhizal Fungi in agriculture soils.

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